

What is claimed:

1. A method for detecting the presence, absence, or quantity of a segmented negative strand RNA virus in a biological specimen suspected of comprising the virus, the method comprising:
 - a) contacting the biological specimen with a genetically engineered vertebrate cell comprising a recombinant RNA molecule that comprises a reporter gene encoding a polypeptide, in which expression of the polypeptide depends upon the presence in the cell of an RNA-dependent RNA polymerase of the virus; and
 - b) detecting the absence, presence, or quantity of the polypeptide encoded by the reporter gene.
2. The method according to claim 1, wherein the segmented negative strand RNA virus is selected from the group consisting of influenza A virus, influenza B virus, and influenza C virus.
3. The method according to claim 1, wherein the RNA molecule is an artificial segment or the complement thereof, the artificial segment further comprising a 5' UTR of a segment and a 3' UTR of a segment, wherein at least one of the 5' UTR and the 3' UTR is a UTR of the virus.
4. The method according to claim 3, wherein the artificial segment comprising the 5' UTR comprises the 5' UTR of the NP segment of an influenza A virus.
5. The method according to claim 3, wherein the 3' UTR of the artificial segment comprises the 3' UTR of the NP segment of an influenza A virus.
6. The method according to claim 1, wherein the polypeptide encoded by the reporter gene is selected from the group consisting of chloramphenicol acetyl transferase,

β -galactosidase, β -glucuronidase, renilla luciferase, firefly luciferase, a green fluorescent protein, and secreted alkaline phosphatase.

7. The method of claim 6, wherein the polypeptide encoded by the reporter gene is selected from the group consisting of a green fluorescent protein and a firefly luciferase.

8. The method according to claim 1, wherein the detecting the absence, presence, or quantity of the polypeptide encoded by the reporter gene comprises detecting the absence, presence, or quantity of a photonic signal emitted by the polypeptide.

9. The method according to claim 1, wherein the genetically engineered vertebrate cell is selected from the group consisting of a 293T kidney cell, an LA-4 cell, a MRC5 cell, an A549 cell, a CV-1 cell, a Vero cell, a LLC-MK1 cell, a HEp2 cell, a 2fTGH cell, a U3A cell, a BHK cell, a monkey primary kidney cell, and a Chinese hamster ovary cell.

10. A method for detecting the presence, absence, or quantity of a segmented negative strand virus in a biological specimen suspected of comprising the segmented negative strand RNA virus, the method comprising:

a) contacting the biological specimen with a genetically engineered vertebrate cell comprising a recombinant DNA molecule that comprises a reporter gene encoding a polypeptide, wherein expression of the polypeptide depends upon the presence in the cell of an RNA-dependent RNA polymerase of the virus; and

b) detecting the absence, presence, or quantity of the polypeptide encoded by the reporter gene.

11. The method according to claim 10, wherein the recombinant DNA molecule comprises, in 5' to 3' order:
- a promoter for a DNA-dependent RNA polymerase;
 - a transcription initiation site for the DNA-dependent RNA polymerase;
 - a DNA sequence encoding an artificial segment; and
 - a transcription termination site.
12. The method according to claim 11, wherein the promoter for a DNA-dependent RNA polymerase is a promoter for RNA polymerase I.
13. The method according to claim 11, wherein the transcription initiation site is a transcription initiation site for RNA polymerase I.
14. The method according to claim 11, wherein the DNA sequence encoding an artificial segment is operably linked to the promoter such that a transcript of the artificial segment is in an anti-sense orientation.
15. The method according to claim 11, wherein the DNA encoding an artificial segment is operably linked to the promoter such that a transcript of the artificial segment is in a sense orientation.
16. The method according to claim 11, wherein the artificial segment comprises a cDNA of a 5' UTR of a segment of a negative strand RNA virus, the reporter gene, and a cDNA of a 3' UTR of a segment of a negative strand RNA virus, wherein at least one of the 3' UTR and the 5' UTR is a UTR of the virus suspected of comprising the biological sample.
17. The method according to claim 16, wherein the at least one of the 3' UTR and the 5' UTR is a UTR of the NP segment of an influenza A virus.

18. The method according to claim 10, wherein the segmented negative strand virus is a virus selected from the group consisting of influenza A virus, influenza B virus, and influenza C virus.

19. The method according to claim 11, wherein the reporter gene encodes a polypeptide selected from the group consisting of chloramphenicol acetyl transferase, β -galactosidase, β -glucuronidase, renilla luciferase, firefly luciferase, a green fluorescent protein, and secreted alkaline phosphatase.

20. The method of claim 26, wherein the polypeptide is selected from the group consisting of a green fluorescent protein and a firefly luciferase.

21. The method according to claim 11, wherein the transcription termination signal is a transcription termination signal for RNA polymerase I.

22. The method according to claim 11, wherein the transcription termination site comprises a sequence encoding a self-cleaving ribozyme.

23. The method according to claim 10, wherein the genetically engineered vertebrate cell is a stably transfected genetically engineered vertebrate cell.

24. The method according to claim 10, wherein the genetically engineered vertebrate cell is a transiently transfected genetically engineered vertebrate cell.

25. The method according to claim 10, wherein the genetically engineered vertebrate cell is a transfected cell selected from the group consisting of a 293T kidney cell, an LA-4 cell, a MRC5 cell, an A549 cell, a CV-1 cell, a Vero cell, a LLC-MK1 cell, a HEp2 cell, a 2fTGH cell, a U3A cell, a BHK cell, a monkey primary kidney cell, and a Chinese hamster ovary cell.

26. A method of determining a differential diagnosis between an infection of influenza A, influenza B, and neither virus in a biological specimen, the method comprising:

- a) contacting the biological specimen with a population of genetically engineered cells, wherein a substantial plurality of the genetically engineered cells comprise a recombinant RNA molecule that comprises a reporter gene encoding a polypeptide, in which expression of the polypeptide depends upon the presence in the cell of an RNA-dependent RNA polymerase of an influenza virus; and
- b) measuring expression of the reporter gene in the population of genetically engineered cells.

27. The method of claim 26, wherein the recombinant RNA is an artificial segment of an influenza A virus, the artificial segment comprising a 5' UTR of a segment of the influenza A virus, an open reading frame of a reporter gene encoding a polypeptide, and a 3' UTR of a segment of the influenza A virus.

28. The method according to claim 26, wherein measuring expression of the reporter gene in the population of genetically engineered cells comprises determining a percentage of cells expressing the reporter gene.

29. The method according to claim 26, wherein the measuring expression of the reporter gene in the population of genetically engineered cells comprises determining mean signal intensity.

30. The method according to claim 26, wherein the reporter gene is selected from the group consisting of chloramphenicol acetyl transferase, β -galactosidase, β -

glucoronidase, renilla luciferase, firefly luciferase, a green fluorescent protein, and secreted alkaline phosphatase.

31. The method according to claim 30, wherein the measuring expression of the reporter gene in the population of genetically engineered cells comprises measuring a photonic signal from a luciferase reporter gene polypeptide or GFP reporter gene polypeptide.

32. The method according to claim 26, wherein the reporter gene encoding the polypeptide is in the sense orientation.

33. The method according to claim 26, wherein the reporter gene encoding the polypeptide is in the anti-sense orientation.

34. The method according to claim 26, wherein the genetically engineered vertebrate cells are selected from the group consisting of 293T kidney cells, LA-4 cells, MRC5 cells, A549 cells, CV-1 cells, Vero cells, LLC-MK1 cells, HEp2 cells, 2fTGH cells, U3A cells, BHK cells, monkey primary kidney cells, and Chinese hamster ovary cells.

35. The method according to claim 26, wherein the substantial plurality of genetically engineered cells comprises at least about 50% of the cells.

36. The method according to claim 26, wherein the substantial plurality of genetically engineered cells comprises at least about 75% of the cells.

37. The method according to claim 26, wherein the substantial plurality of genetically engineered cells comprises at least about 90% of the cells.

38. A method for detecting the presence, absence, or quantity of a first segmented negative strand RNA virus and a second segmented negative strand RNA virus in a biological specimen, the method comprising:

a) contacting the biological specimen with a genetically engineered vertebrate cell, the cell comprising a first recombinant RNA molecule that comprises a first reporter gene in which expression depends upon the presence in the cell of an RNA-dependent RNA polymerase of a first segmented negative strand virus, and a second recombinant RNA molecule that comprises a second reporter gene in which expression depends upon the presence in the cell of an RNA-dependent RNA polymerase of a second segmented negative strand virus; and

b) detecting the absence, presence, or quantity of a first polypeptide encoded by the first reporter gene and the absence, presence, or quantity a second polypeptide encoded by the second reporter gene.

39. The method of claim 38, wherein the first recombinant RNA molecule is a first artificial segment comprising a 5' UTR of the first segmented negative strand RNA virus, an open reading frame of the first reporter gene, and a 3' UTR of the first segmented negative strand RNA virus, and wherein the second recombinant RNA molecule is a second artificial segment comprising a 5' UTR of the second segmented negative strand RNA virus, an open reading frame of the second reporter gene, and a 3' UTR of the second segmented negative strand RNA virus.

40. The method according to claim 38, wherein the first segmented negative strand RNA virus and the second segmented negative strand RNA virus are each selected

from the group consisting of an influenza A virus, an influenza B virus, and an influenza C virus.

41. The method according to claim 44, wherein the first reporter gene and the second reporter gene each encode a polypeptide selected from the group consisting of chloramphenicol acetyl transferase, β -galactosidase, β -glucuronidase, renilla luciferase, firefly luciferase, a green fluorescent protein, and secreted alkaline phosphatase.

42. The method of claim 41, wherein the first reporter gene and the second reporter gene each encode a polypeptide selected from the group consisting of firefly luciferase, renilla luciferase and a green fluorescent protein.

43. The method according to claim 38, wherein the open reading frame of at least one of the first reporter gene and the second reporter gene is in the anti-sense orientation.

44. The method according to claim 38, wherein the open reading frame of at least one of the first reporter gene and the second reporter gene is in the sense orientation.

45. The method according to claim 38, wherein the genetically engineered vertebrate cell is a transfected cell selected from the group consisting of a 293T kidney cell, an LA-4 cell, a MRC5 cell, an A549 cell, a CV-1 cell, a Vero cell, a LLC-MK1 cell, a HEp2 cell, a 2fTGH cell, a U3A cell, a BHK cell, a monkey primary kidney cell, and a Chinese hamster ovary cell.

46. A method for detecting the presence, absence, or quantity of a first segmented negative strand RNA virus and a second segmented negative strand RNA virus in a biological specimen suspected of comprising the first or second segmented negative strand RNA virus, the method comprising:

a) contacting the biological specimen with a genetically engineered vertebrate cell comprising a first recombinant DNA molecule that comprises a first reporter gene in which expression depends upon the presence in the cell of a viral RNA of the first segmented negative strand virus, and a second recombinant DNA molecule that comprises a second reporter gene in which expression depends upon the presence in the cell of a viral RNA of the second segmented negative strand virus; and

b) detecting the absence, presence, or quantity of a polypeptide encoded by the first and second reporter gene.

47. The method according to claim 46, wherein the first recombinant RNA molecule comprises, in 5' to 3' order:

a) a promoter for a DNA-dependent RNA polymerase;

b) a transcription initiation site for the DNA-dependent RNA polymerase;

c) a first artificial segment, wherein the first artificial segment comprises a cDNA of a 5' UTR of a segment of a first segmented negative strand RNA virus, a DNA sequence of an open reading frame of a first reporter gene, and a cDNA of a 3' UTR of a segment of the first segmented negative strand RNA virus;

d) a transcription termination site for the DNA-dependent RNA polymerase; and wherein the second recombinant RNA molecule comprises, in 5' to 3' order:

e) a promoter for the DNA-dependent RNA polymerase;

f) a transcription initiation site for the DNA-dependent RNA polymerase;

g) a second artificial segment, wherein the second artificial segment comprises a cDNA of a 5' UTR of a segment of a second segmented negative strand RNA virus, a DNA sequence of an open reading frame of a second reporter gene, and a cDNA of a 3' UTR of a segment of the second segmented negative strand RNA virus; and

h) a transcription termination site for the DNA-dependent RNA polymerase

48. The method according to claim 46, wherein the first segmented negative strand RNA virus and the second segmented negative strand RNA virus are each selected from the group consisting of an influenza A virus, an influenza B virus, and an influenza C virus.

49. The method according to claim 47, wherein each promoter for a DNA-dependent RNA polymerase is a promoter for RNA polymerase I.

50. The method according to claim 47, wherein each transcription initiation site for the DNA-dependent RNA polymerase is a transcription initiation site for RNA polymerase I.

51. The method according to claim 47, wherein each transcription termination site comprises a sequence encoding a self-cleaving ribozyme.

52. The method according to claim 47, wherein each transcription termination site is an RNA polymerase I termination site.

53. The method according to claim 46, wherein the genetically engineered vertebrate cell comprises a recombinant DNA molecule comprising:

a first promoter for a DNA-dependent RNA polymerase;

a first transcription initiation site for the DNA-dependent RNA polymerase;

a first DNA sequence encoding a first artificial segment, the first artificial segment comprising a first 5' UTR and a first 3' UTR of a first segmented negative strand RNA virus, and an open reading frame of a first reporter gene;

a first transcription termination site;

a second promoter for a DNA-dependent RNA polymerase;

a second transcription initiation site for the DNA-dependent RNA polymerase;

a second DNA sequence encoding a second artificial segment, the second artificial segment comprising a second 5' UTR and a second 3' UTR of a second segmented negative strand RNA virus, and an open reading frame of a second reporter gene; and

a second transcription termination site.

54. The method according to claim 46, wherein the genetically engineered vertebrate cell is a stably transfected genetically engineered vertebrate cell.

55. The method according to claim 46, wherein the genetically engineered vertebrate cell is a transiently transfected genetically engineered vertebrate cell.

56. The method according to claim 46, wherein the genetically engineered vertebrate cell is a transfected cell selected from the group consisting of a 293T kidney cell, an LA-4 cell, a MRC5 cell, an A549 cell, a CV-1 cell, a Vero cell, a LLC-MK1 cell, a HEp2 cell, a 2fTGH cell, a U3A cell, a BHK cell, a monkey primary kidney cell, and a Chinese hamster ovary cell.

57. A kit for detecting the presence, absence, or quantity of a segmented negative strand RNA virus, the kit comprising instructions and an isolated recombinant DNA molecule, the isolated recombinant molecule comprising a reporter gene in which

expression in a cell transfected with the molecule depends upon the presence in the cell of an RNA-dependent RNA polymerase of the segmented negative strand virus.

58. The kit of claim 57, wherein the isolated recombinant DNA molecule comprises, in 5' to 3' order:

- a) a promoter for a DNA-dependent RNA polymerase;
- b) a transcription initiation site for the DNA-dependent RNA polymerase;
- c) a sequence encoding an artificial segment comprising a DNA sequence comprising an open reading frame of a reporter gene encoding a polypeptide; and
- d) a transcription termination site for the DNA-dependent RNA polymerase.

59. The kit of claim 57, wherein the segmented negative strand RNA virus is selected from the group consisting of influenza A virus, influenza B virus, and influenza C virus.

60. The kit of claim 57, wherein the promoter for a DNA-dependent RNA polymerase is a promoter for RNA polymerase I.

61. The kit claim 57, wherein the transcription initiation site for the DNA-dependent RNA polymerase is a transcription initiation site for RNA polymerase I.

62. The kit of claim 57, wherein the artificial segment further comprises a cDNA of a 5' UTR of a segment of the segmented negative strand RNA virus and a cDNA of a 3' UTR of a segment of the segmented negative strand RNA virus.

63. The kit of claim 62, wherein at least one of the cDNA of the 5' UTR of a segment of the segmented negative strand RNA virus and the cDNA of the 3' UTR of a segment of the segmented negative strand RNA virus is a cDNA of a UTR of an NP segment of an influenza A virus.

64. The kit of claim 57, wherein the DNA sequence of an open reading frame of a reporter gene encodes a polypeptide selected from the group consisting of chloramphenicol acetyl transferase, β -galactosidase, β -glucuronidase, renilla luciferase, firefly luciferase, a green fluorescent protein, and secreted alkaline phosphatase.

65. The kit of claim 64, wherein the polypeptide is selected from the group consisting of a green fluorescent protein and a firefly luciferase.

66. The kit of claim 57, wherein the DNA sequence of an open reading frame of a reporter gene is in the anti-sense orientation.

67. The kit of claim 57, wherein the DNA sequence of an open reading frame of a reporter gene is in the sense orientation.

68. The kit of claim 57, wherein the transcription termination signal is a transcription termination signal for RNA polymerase I.

69. The kit of claim 57, wherein the transcription termination signal comprises a ribozyme sequence.

70. The kit of claim 57, wherein the kit further comprises a vertebrate cell.

71. The kit of claim 57, wherein the vertebrate cell is a mammalian cell.

72. The kit of claim 71, wherein the vertebrate cell is selected from the group consisting of a 293T kidney cell, an LA-4 cell, a MRC5 cell, an A549 cell, a CV-1 cell, a

Vero cell, a LLC-MK1 cell, a HEp2 cell, a 2fTGH cell, a U3A cell, a BHK cell, a monkey primary kidney cell, and a Chinese hamster ovary cell.

73. The kit of claim 72, wherein the wherein the isolated recombinant DNA molecule is comprised by the vertebrate cell.

74. The kit of claim 80, wherein the vertebrate cell is selected from the group consisting of a 293T kidney cell, an LA-4 cell, a MRC5 cell, an A549 cell, a CV-1 cell, a Vero cell, a LLC-MK1 cell, a HEp2 cell, a 2fTGH cell, a U3A cell, a BHK cell, a monkey primary kidney cell, and a Chinese hamster ovary cell.

75. A quantitative screen for an anti-viral drug directed against a segmented negative strand RNA virus, the screen comprising:

a) contacting a candidate anti-viral compound with a genetically engineered cell or cell population, the cell or cell population comprising a recombinant RNA molecule comprising a reporter gene encoding a polypeptide for which expression depends upon the presence in the cell of an RNA-dependent RNA polymerase of the virus, and the cell or cell population further comprising an RNA-dependent RNA polymerase of the segmented negative strand RNA virus; and

b) detecting the quantity of the polypeptide produced by the cell or cell population.

76. The quantitative screen for an anti-viral drug according to claim 75, wherein the segmented negative strand RNA virus is selected from the group consisting of influenza A virus, influenza B virus, and influenza C virus.

76. The quantitative screen for an anti-viral drug according to claim 75, wherein the recombinant RNA molecule is an artificial segment further comprising a 5'

UTR of a segment of the segmented negative strand RNA virus and a 3' UTR of a segment of the segmented negative strand RNA virus.

77. The quantitative screen for an anti-viral drug according to claim 76, wherein the 5' UTR of the artificial segment comprises the 5' UTR of an NP segment of an influenza A virus.

78. The quantitative screen for an anti-viral drug according to claim 76, wherein the 3' UTR of the artificial segment comprises the 3' UTR of an NP segment of an influenza A virus.

79. The quantitative screen for an anti-viral drug according to claim 75, wherein the polypeptide encoded by the reporter gene is selected from the group consisting of chloramphenicol acetyl transferase, β -galactosidase, β -glucuronidase, renilla luciferase, firefly luciferase, a green fluorescent protein, and secreted alkaline phosphatase.

80. The quantitative screen for an anti-viral drug according to claim 75, wherein the polypeptide encoded by the reporter gene is selected from the group consisting of a green fluorescent protein and a firefly luciferase, and wherein the detecting the quantity of the polypeptide encoded by the reporter gene comprises detecting the quantity of a photonic signal emitted by the polypeptide or a substrate thereof.

81. The quantitative screen for an anti-viral drug according to claim 75, wherein the genetically engineered vertebrate cell is a stably transfected genetically engineered vertebrate cell, wherein the cell further comprises a recombinant DNA encoding the recombinant RNA.

82. The quantitative screen for an anti-viral drug according to claim 75, wherein the genetically engineered vertebrate cell is a transiently transfected genetically engineered vertebrate cell, wherein the cell further comprises a recombinant DNA encoding the recombinant RNA.

83. The quantitative screen for an anti-viral drug according to claim 75, wherein the genetically engineered vertebrate cell is a transfected cell selected from the group consisting of a 293T kidney cell, an LA-4 cell, a MRC5 cell, an A549 cell, a CV-1 cell, a Vero cell, a LLC-MK1 cell, a HEp2 cell, a 2fTGH cell, a U3A cell, a BHK cell, a monkey primary kidney cell, and a Chinese hamster ovary cell.

84. A quantitative screen for an anti-viral drug directed against a segmented negative strand RNA virus, the screen comprising:

contacting a candidate anti-viral compound with a first cell, the first cell susceptible to proliferative infection by the virus;

infecting the first cell with the segmented negative strand RNA virus, thereby providing a production sample;

contacting a second cell with the production sample, wherein the second comprises a recombinant RNA molecule comprising a reporter gene encoding a polypeptide for which expression depends upon the presence in the cell of an RNA-dependent RNA polymerase of the virus; and

detecting the quantity of the polypeptide produced by the second cell.

85. The quantitative screen for an anti-viral drug according to claim 84, wherein the segmented negative strand RNA virus is selected from the group consisting of an influenza A virus, an influenza B virus, and an influenza C virus.

86. The quantitative screen for an anti-viral drug according to claim 84, wherein the recombinant RNA molecule is an artificial segment further comprising a 5' UTR of a segment of the segmented negative strand RNA virus and a 3' UTR of a segment of the segmented negative strand RNA virus.

87. The quantitative screen for an anti-viral drug according to claim 86, wherein the 5' UTR of the artificial segment comprises the 5' UTR of an NP segment of an influenza A virus.

89. The quantitative screen for an anti-viral drug according to claim 86, wherein the 3' UTR of the artificial segment comprises the 3' UTR of an NP segment of an influenza A virus.

90. The quantitative screen for an anti-viral drug according to claim 84, wherein the polypeptide encoded by the reporter gene is selected from the group consisting of chloramphenicol acetyl transferase, β -galactosidase, β -glucuronidase, renilla luciferase, firefly luciferase, a green fluorescent protein, and secreted alkaline phosphatase.

91. The quantitative screen for an anti-viral drug according to claim 84, wherein the polypeptide encoded by the reporter gene is selected from the group consisting of a green fluorescent protein and a firefly luciferase, and wherein the detecting the quantity of the polypeptide encoded by the reporter gene comprises detecting the quantity of a photonic signal emitted by the polypeptide or a substrate thereof.

92. The quantitative screen for an anti-viral drug according to claim 84, wherein the genetically engineered vertebrate cell is a stably transfected genetically

engineered vertebrate cell, wherein the cell further comprises a recombinant DNA encoding the recombinant RNA.

93. The quantitative screen for an anti-viral drug according to claim 84, wherein the genetically engineered vertebrate cell is a transiently transfected genetically engineered vertebrate cell, wherein the cell further comprises a recombinant DNA encoding the recombinant RNA.

94. The quantitative screen for an anti-viral drug according to claim 84, wherein the genetically engineered vertebrate cell is a transfected cell selected from the group consisting of a 293T kidney cell, an LA-4 cell, a MRC5 cell, an A549 cell, a CV-1 cell, a Vero cell, a LLC-MK1 cell, a HEp2 cell, a 2fTGH cell, a U3A cell, a BHK cell, a monkey primary kidney cell, and a Chinese hamster ovary cell.